

### **REMARKS**

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein, is respectfully requested. Claim 36 is amended. Claims 37-43 are cancelled without prejudice or disclaimer. Claim 44 is newly added. The pending claims are claims 36 and 44.

Claims 37-43 are cancelled solely to advance prosecution, and Applicants reserve the right to reintroduce them in a later filed divisional application.

New claim 44 is fully supported by the specification as originally filed, and no new subject matter has been added. Support for newly added claim 44 can be found, for example, in the specification at page 8, lines 6-8.

Support for the amendment to claim 36 is found in the specification at page 5, lines 7-14.

### **The 35 U.S.C. § 112 Rejections of the Claims**

The Examiner rejected claims 38-43 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The cancellation of claims 38-43 renders this rejection moot. The Examiner also rejected claims 36-38 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. In particular, the Examiner asserts that the specification, at page 5, line 7, does not provide support for the phrase “wherein the shuttle plasmid is present in an amount of about fifteen times molar ratio as compared to an amount of the backbone plasmid” in claim 36, and therefore the phrase constitutes new matter. Applicant disagrees with the Examiner’s assertion, but has deleted this phrase from the claims to expedite prosecution; therefore, this rejection is overcome. As this rejection may be maintained with respect to the pending claims, it is respectfully traversed.

As amended, claim 36 is directed to a method for producing recombinant adenovirus consisting of transfecting a host cell with an Ad backbone plasmid comprising an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR,

and a shuttle plasmid comprising Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome, wherein the host cell is transfected with more molecules of the shuttle plasmid than molecules of the backbone plasmid.

As evidence that new matter has not been introduced into the claims, the Examiner is respectfully requested to consider the Rule 132 Declaration of Richard D. Anderson and Ronald E. Haskell ("the Declaration"), attached hereto. In the Declaration, Richard D. Anderson and Ronald E. Haskell, two of the co-inventors of the present application, state that the above-identified application discloses a cloning system for the rapid generation of recombinant adenoviral vectors. They further state that recombinant adenoviral (Ad) vectors are frequently used to deliver cloned DNA into cells or animals, *e.g.*, *in vitro* gene transfer, *in vivo* vaccination and gene therapy (Davidson and Roessler, Adenoviral-Mediated Gene Transfer: Potential Therapeutic Applications, Chapter 11 in Viral Vectors: Gene Therapy and Neuroscience Applications, Kaplitt and Loewy, *eds.*, Academic Press, San Diego (1995) (Exhibit A).

In the Declaration, Richard D. Anderson and Ronald E. Haskell state that Adenovirus type 5 (Ad5) has been used extensively for the production of recombinant adenoviral vectors (Kozarsky and Wilson, Current Opinion in Genetics and Development, 3, 499-503 (1993) (Exhibit B). They further state that the adenovirus genome consists of linear, 36 Kb double-stranded DNA, and that by convention, the genome is divided into 100 map units (m.u.), *i.e.*, 10 m.u. equals 3600 bp. In addition, they state that the adenoviral genome has been completely sequenced (see GenBank accession no. M73260 (Ad5)) and well characterized. For example, the adenoviral genome contains inverted terminal repeats (ITRs) at each end, and the adenoviral gene products are organized into early (E1 through E4) and late (L1 through L5) regions. Moreover, they state that it is known that the 0-1 m.u. region contains the left hand ITR (1-103 bp) and packaging signals (194-358 bp) of the adenoviral genome. The E1 region is known to contain two genes, specifically E1A, located at 468-1676 bp, and E1B, located at 2016-3503 bp. The E3 region is known to be located at 27,609-30,864 bp.

Richard D. Anderson and Ronald E. Haskell further state in the Declaration that dl309 is a biologically selected, restriction enzyme-site-loss variant of wild-type Ad5 (Jones and Shenk, Cell, 17, 683-689 (1979) (Exhibit C). dl309 is identical to Ad5 except for the changes reported by Jones and Shenk (1979), and as shown in GenBank accession no. U22898 (Exhibit D). That

is, Richard D. Anderson and Ronald E. Haskell state that dl309 is approximately 36 Kb in size, and the Ad5 backbone vectors of the above-identified application are based upon dl309 (page 8, line 21 of the specification).

In addition, Richard D. Anderson and Ronald E. Haskell state that Figure 1 of the above-identified specification illustrates the generation of pacAd5 9.2-100, an exemplary backbone plasmid of the invention. They state that it is clear from Figure 1 that pacAd5 9.2-100 has a polyadenylation site (abbreviated as "pA"), the 9.2-100 m.u. of Ad5 genomic DNA, a PacI restriction site that flanks the 5' end of the pA signal, a PacI restriction site that flanks the 3' end of the Ad5 genomic DNA, and additional plasmid DNA that contains an ampicillin resistance gene (abbreviated as "Amp<sup>r</sup>") with a ScaI site. Many suitable plasmids were well-known in the art, such as pBR322, which is 4.3 Kb, and pUC19, which is 2.7 Kb. Therefore, Richard D. Anderson and Ronald E. Haskell state that it would be clear to an art worker that a backbone plasmid of the invention includes approximately 33 Kb of Ad5 genomic DNA and about 3-4 Kb of plasmid DNA. Thus, one of ordinary skill in the art, at the time the application was filed, would have been aware that the backbone plasmids of the invention were approximately 36 Kb.

In the Declaration, Richard D. Anderson and Ronald E. Haskell state that standard shuttle plasmids, *i.e.*, those routinely used to co-transfect HEK293 cells, are useful in the generation of recombinant adenovirus using the present invention (page 6, lines 29-30 and page 8, lines 6-7 of the above-identified application). At the time the above-identified application was filed, there were many standard shuttle plasmids well-known in the field of recombinant adenovirus generation. For example, Richard D. Anderson and Ronald E. Haskell state that several shuttle plasmids based upon the pAdBglII shuttle plasmid were commonly employed and reported in the literature prior to the filing date of the present application (Davidson et al., Nat. Genetics, 3, 219-223 (1993); Davidson et al., Experimental Neurology, 125, 258-267 (1994) and Aoki et al., Molecular Medicine, 5, 224-231 (1999)). When empty, *i.e.*, devoid of a subcloned cDNA of interest, pAdBglII is approximately 5.8 Kb. A copy of a cartoon depicting pAdBglII is showing in Exhibit E of the Declaration.

In the Declaration, Richard D. Anderson and Ronald E. Haskell state that additional examples of shuttle plasmids that were known as of the filing date of the present application and that are useful in the production of recombinant adenovirus can be found in U.S. Patent

5,922,576 (He et al., referred to as "the '576 patent" (of record)). Figure 2 of the '576 patent discloses four shuttle plasmids that range in size from approximately 6.7 Kb to 9.2 Kb (when empty).

Richard D. Anderson and Ronald E. Haskell further declare that the function of a shuttle plasmid is to transfer cDNAs of various sizes from the shuttle vector into a viral construct (page 6, lines 19-20 of the specification). Due to the packaging capacity of adenovirus, the range of cDNAs that can be inserted into the recombinant adenovirus using the cloning system of the invention is 0-10,000 bp. Therefore, Richard D. Anderson and Ronald E. Haskell state that the art worker, at the time the present application was filed, would have been aware that shuttle plasmids useful in the present invention could range in size from approximately 6 Kb to approximately 20 Kb.

The Examiner is respectfully requested to consider the following calculations, which establish that new matter has not been introduced into the claims. By convention, 1 DNA base pair (bp) equals 660 daltons (Da) or 660 grams/mole (see, for example, page 119 of Stryer, Biochemistry, W.H. Freeman and Company, New York (3<sup>rd</sup> edition, 1988)(a copy is attached hereto)).

To determine the range of the number of molecules of shuttle plasmid that a host cell is transfected with according to the method of claim 36, the art worker can multiply the size of shuttle plasmid present, *i.e.*, 6Kb-20Kb, by 660 grams/mole, *e.g.*, (6000 bp)·(660 Da/bp) = 3.96 x 10<sup>6</sup> grams/mole to (20,000bp)·(660 Da/bp) = 1.32 x 10<sup>7</sup> grams/mole. Page 5, lines 7 of the specification discloses, for example, that the HEK293 cells were co-transfected with 15 µg of the shuttle plasmid, *i.e.*, a host cell can be transfected with (15 x 10<sup>-6</sup> grams)·(1 mole/3.96 x 10<sup>6</sup> grams) = 3.79 x 10<sup>-12</sup> moles of shuttle to (15 x 10<sup>-6</sup> grams)·(1 mole/1.32 x 10<sup>7</sup> grams) = 1.14 x 10<sup>-12</sup> moles of shuttle. In other words, the host cell can be transfected with (3.79 x 10<sup>-12</sup> moles)·(6.02 x 10<sup>23</sup> molecules/mole) = 2.28 x 10<sup>12</sup> molecules of shuttle plasmid/reaction to (1.14 x 10<sup>-12</sup> moles)·(6.02 x 10<sup>23</sup> molecules/mole) = 6.86 x 10<sup>11</sup> molecules of shuttle plasmid/reaction.

To determine the number of molecules of backbone plasmid present, which is about 36Kb, page 5, line 7 of the specification discloses that 4 µg were used to transfect HEK293 cells, *i.e.*, (4 x 10<sup>-6</sup> grams)·(1 mole/2.376 x 10<sup>7</sup> grams) = 1.7 x 10<sup>-13</sup> moles; which is (1.7 x 10<sup>-13</sup> moles)·(6.02 x 10<sup>23</sup> molecules/mole) = 1.01 x 10<sup>11</sup> molecules of backbone plasmid/reaction.

Thus, it is clear that according to the claimed method, there can be more than 20 times as many molecules of shuttle plasmid present in a transfection reaction as compared to the number of molecules of backbone plasmid. Therefore, Applicants' specification clearly provides support for claims 36 and 44 as presently claimed.

Hence, it is clear that Applicants were in possession of the subject matter of the pending claims as of the filing date of the present application and that new matter has not been added. Withdrawal of the new matter rejection is therefore respectfully requested.

*The 35 U.S.C. §103 Rejection of the Claims*

The Examiner rejected claims 36 and 37 under 35 U.S.C. § 103(a) as being unpatentable over Aoki *et al.*, Molecular Medicine, 5, 224-231 (1999) and He *et al.* (U.S. Patent No. 5,922,576). It is Applicant's understanding that the examiner is citing Aoki *et al.* as a primary reference and He *et al.* as a secondary reference. Since this rejection is not clear, Applicant sets forth remarks for each reference individually, and also in combination.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed method. Second, the art must provide a reasonable expectation of success. Finally, the prior art references must teach or suggest all the claim limitations (M.P.E.P. § 2143). The teaching or suggestion to arrive at the claimed method and the reasonable expectation of success must both be found in the prior art, not in Applicant's disclosure (M.P.E.P. § 2143, *citing with favor*, In re Vaeck, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

The pending claims are directed to a method for producing recombinant adenovirus by transfecting or contacting a host cell with (a) an Ad backbone plasmid comprising an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR, and (b) a shuttle plasmid comprising Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome, wherein the host cell is transfected or contacted with more molecules of the shuttle plasmid than molecules of the backbone plasmid.

Aoki et al. disclose the *in vitro* site-specific recombination of equal moles of viral and plasmid DNAs using in a cell-free reaction mixture (pages 225 and 228 of Aoki et al.). Following the recombination event, Aoki et al. disclose the transfection of 293 cells with the purified, recombined DNA (page 225 and Figure 1A of Aoki et al.). There is nothing in Aoki et al. that teaches or suggests method for producing recombinant adenovirus by transfecting or contacting a host cell with Applicants' claimed Ad backbone and shuttle plasmids, let alone such a method wherein the host cell is transfected with more molecules of the shuttle plasmid than molecules of the backbone plasmid. Thus, Aoki et al. do not teach all of the recited elements of the claims.

The Examiner cited to He et al. in combination with Aoki et al. because Aoki et al. "do not teach a transfecting a host cell by calcium phosphate." (Office Action at pages 5-6.) Claim 37 has now been cancelled, and the remaining claims do not recite calcium phosphate transfection. Thus, this rejection has been rendered moot. Further, the plasmids taught by He et al. are different than those recited in the pending claims. Therefore, the pending claims are not obvious over He et al.

Insofar as a combination of Aoki et al. and He et al. may be applied to the pending claims, Applicant asserts that one of skill in the art would not have combined these references to create the present methods. When finding a claimed invention obvious, the references relied on must be considered as a whole, and must also suggest the desirability of making the combination. *Lindemann Maschinefabrik GmbH v. American Hoist and Derrick Co.*, 221 USPQ 481, 488 (Fed. Cir. 1984). Furthermore, "[w]e do not 'pick and choose among the individual elements of assorted prior art references to recreate the claimed invention' but rather, we look for 'some teaching or suggestion in the references to support their use in the particular claimed combination.'" *Symbol Tech., Inc. v. Opticon, Inc.*, 19 USPQ2d 1241, 1246 (Fed. Cir. 1991) (quoting *Smithkline Diagnostics, Inc. v. Helena Lab. Corp.*, 8 USPQ2d 1468, 1475 (Fed. Cir. 1988)); see also, *In re Sang Su Lee*, 61 U.S.P.Q.2d 1430-1436, 1433 (Fed. Cir. 2002).

Aoki et al. and He et al. teach two entirely different cloning methods. As discussed previously, Aoki et al. discloses a multi-step process: (1) in a cell-free reaction mixture equal moles of shuttle plasmid and adenoviral cosmid were recombined *in vitro* for 3 hours at 37°C along with Cre recombinase (except in the negative control), (2) the reaction mixture was

inactivated at 70°C for 5 minutes, (3) DNA was purified using a plasmid purification kit, and (4) the DNA was transfected into 293 cells. Admittedly, their negative control exhibited a residual amount of homologous recombination, but even this negative control was subjected to the four steps.

He *et al.* discuss a very different cloning method from either Aoki *et al.* or the present application. As discussed above, cloning using Ad vectors has been long-studied, and several difficulties were well known. He *et al.* worked to overcome these difficulties by preparing very specific Ad backbone vectors. They produced recombinant adenovirus using adenoviral vectors with Ad5 sequence except nucleotides 1-3,533 (which corresponds to approximately 0-9.8 m.u.), and 28,130-30,820 (which corresponds to approximately 78-86 m.u.), and possibly also deleted 32,816-35,462 (column 8, lines 32-38). He *et al.* further disclose that a “key step” to the production of adenoviral plasmids is the co-transformation of the linear DNA molecule and the supercoiled adenoviral vector into bacteria, for example, by employing any number of transformation methods such as by use of calcium phosphate solutions (column 4, lines 20-30).

There is no teaching in He *et al.* that other backbone plasmids could be substituted for the ones that they used. Therefore, one of skill in the art would not have been motivated to substitute any other Ad backbone plasmids (such as the plasmids used in the negative control of Aoki *et al.*) with the transfection method of He *et al.* to generate recombinant cloning system. Further, one of ordinary skill in the art would not have had a reasonable expectation of success unless one used the backbones optimized by He *et al.* Thus, when the cited references are considered as a whole, they do not suggest the desirability of making the combination. With all due respect, Applicant disagrees with the comments of the examiner on page 6, second paragraph, of the Office Action. For the reasons stated above, one skilled in the art would not have been motivated to combine the cited references. Further, one of skill would not have had a reasonable expectation that one could successfully carry out the co-transfection of the Ad vectors of Aoki *et al.* with the transfection method of He *et al.* to generate recombinant Ad in a one-step process for *in vivo* assembly.

In view of the above amendments and remarks, withdrawal of the 35 U.S.C. § 103 rejection is respectfully requested.

Conclusion

Applicants respectfully submit that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6961) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743


Respectfully submitted,

BEVERLY L. DAVIDSON ET AL.,

By their Representatives,

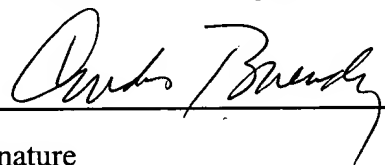
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